LESSON 12:
PRACTICAL APPLICATIONS OF SOMATIC EMBRYOGENESIS

Objective

The objective will be to know the various fields where somatic embryogenesis has been utilized for plant regeneration and transformation. The students will be able to gather information on practical applications of this method.

Somatic Embryogenesis has following practical applications:

Clonal Propagation

Somatic embryogenesis has a potential application in plant improvement. Since both the growth of embryogenic cells and subsequent development of somatic embryos can be carried out in a liquid medium, it is possible to combine somatic embryogenesis with engineering technology to create large-scale mechanised or automated culture systems. Such systems are capable of producing propagules (somatic embryos) repetitively with low labour inputs. In this process of repetitive somatic embryogenesis (also referred to as accessory, adventive, or secondary somatic embryogenesis) a cycle is initiated whereby somatic embryos proliferate from the previously existing somatic embryo in order to produce clones.

Cloning Zygotic Embryos for Repetitive Somatic Embryogenesis

A wide range of soybean genotypes have been tested for their ability to undergo auxin-stimulated somatic embryogenesis during cloning of zygotic embryos (Barwale et al. 1986; Komatsuda and Ohyama 1988). All of them are reported to form somatic embryos provided appropriate nutrients are provided in the medium. The role of genotypes in conferring regeneration capacity is further supported by studies on zygotic embryo cloning of wheat, rice and maize. Analysis of various cultivars demonstrated that the regeneration capacity of these crops was directly affected by non-additive, additive and cytoplasmic factors. However, the genotype which has the capacity to undergo repetitive somatic embryogenesis can be back-crossed to elite lines in order to transform the latter with capacity for high regeneration of somatic embryos. Such a type of transformation could play an important role in plant breeding since through in vitro techniques high quality somatic embryos have been produced in 80 species of tropical crops.

Raising Somaclonal Variants in Tree Species

Embryos formed directly from pre embryogenic cells appear to produce relatively uniform clonal material, whereas the indirect pathway generates a high frequency of somaclonal variants. Mutation during adventive embryogenesis may give rise to a mutant embryo which on germination would form a new strain of plant. Nucellar embryos, like shoot tips, are free of virus and can be used for raising virus-free clones, especially from some tree species (e.g., poly embryonate Citrus) where shoot tip culture has not been successful. For clonal propagation of tree species, somatic embryogenesis from nucellar cells may offer the only rapid means of obtaining juvenile plants equivalent to seedlings with parental genotype. Clonal propagation through somatic embryogenesis has been reported in 60 species of woody trees representing 25 families. Somatic embryos with genotype of a selected elite parent are potentially convenient organs for cryopreservation and germplasm storage.

Synthesis of Artificial Seeds

There is considerable worldwide interest in the development of methods for encapsulation of somatic embryos to enable them to be sown under field conditions as 'synthetic' or 'artificial' seeds. Research programmes on production of artificial seeds via somatic embryogenesis in respect of commercially important crops would not only contribute to increased agricultural production, but also add to our basic knowledge of the regulatory mechanisms which control plant growth and differentiation.

What is an Artificial / Synthetic Seed?

An artificial seed is a bead of gel containing a somatic embryo (or shoot bud), and the nutrients, growth regulators, pesticides, antibiotics etc. needed for the development of a complete plantlet from the enclosed SE / shoot bud.

Artificial seeds, consisting of somatic embryos enclosed in a protective coating, have been proposed as a 'low cost – high volume' propagation system. The inherent advantages of artificial seeds are the production of many somatic embryos and the use of conventional seed.handling techniques for embryo delivery. The objective is to produce clonal 'seeds' at a cost comparable to true seeds.

Two types of artificial seeds have been developed, namely, hydrated and desiccated.

Redenbergh et al. (1986) developed hydrated artificial seeds by mixing somatic embryos of alfalfa, celery and cauliflower with sodium alginate, followed by dropping into a solution of calcium chloride to form calcium-alginate beads. The beads become hardened as calcium alginate is formed. After about 20-30 min. the artificial seeds are removed, washed with water and used for planting. Hydrated artificial seeds are sticky and difficult to handle on a large scale, dry rapidly in the open air. These problems can be resolved by providing a waxy coating over the beads. However, it is not possible to store, except at low temperatures and for limited periods, hydrated artificial seeds and they have to be planted soon after they are produced. Precision machines for large scale encapsulation of SEs have been devised.

Kim and Janick (1989) applied synthetic seed coats to clumps of carrot somatic embryos to develop desiccated artificial seeds. They mixed equal volumes of embryo suspension and 5% solution of polyethylene oxide (polyox WSR N-750), a water-soluble resin, which subsequently dried to form polyembryonic desiccated wafers. The survival of encapsulated embryos was further achieved by embryo hardening' treatments with 12%

sucrose or10-6M ABA, followed by chilling at high inoculum density.

Another delivery system for somatic embryos for obtaining transgenic plants is Fluid- drilling. Embryos are suspended in a viscous-carrier gel which extrudes into the soil. The primary problem in fluid-drilling is that the sucrose level necessary to permit conversion also promotes rapid growth of contaminating micro-organisms in a non-aseptic system. Gray (1987) found that somatic embryos of orchard grass (Dactylis glomerata) became quiescent when desiccated in empty plastic petri dishes at 70% relative humidity at 23°C which amounted to loss of 13% water. However, after 21 days of storage, desiccated embryos when rehydrated in vitro germinated to produce viable plantlets though limited (4%) in number. Senaratna et al (1990) treated alfalfa somatic embryos with ABA at the torpedo to cotyledonary stages in order to increase their tolerance to desiccation. Over 60% of such desiccated embryos germinated when placed on a moist filter paper or sown directly onto sterile soil and formed plantlets. Heat-shock treatments also induced a degree of desiccation tolerance comparable to that conferred by ABA treatment and had no detrimental effect on the subsequent growth of the plantlets.

In general, the gels contain inorganic nutrients, a carbon source, fungicides, bactericides and other growth promoting substances necessary for SE germination and seedling establishment in the field. When shoot buds are encapsulated, growth regulators needed for rooting and shoot growth are also included. The SE must be of good quality to be able to germinate in a high frequency under field/ greenhouse conditions. Alfalfa is the most extensively investigated plant species in this regard. Upto 50% of the artificial seeds germinate in greenhouse and the technology has not yet been commercialized.

Source of Regenerable Protoplast System

Embryogenic callus, suspension cultures and somatic embryos have been employed as sources of protoplast isolation for a range of species. Cells or tissues in these systems have demonstrated the potentiality to regenerate in cultures and, therefore, yield protoplasts that are capable of forming whole plants. Embryogenic cultures are especially valuable in providing a source of regenerable protoplasts in the graminaceous, coniferous and citrus species. Attempts to achieve regeneration of callus or even sustained divisions in mesophyll-derived protoplasts of Gramineae proved unsuccessful until Vasil and Vasil (1980) turned to embryogenic cultures obtained from immature embryos of pearl millet (Pennisetum purpureum) as the source of protoplasts. Protoplasts from these cultures were induced to divide to form a cell mass from which embryoids, and even plantlets, regenerated on a suitable nutrient medium. Similar success was subsequently reported by other workers with embryogenic suspension of Panicum maximum, Pennisetum purpureum, Qryza sativa, Saccharum officinarum, Lolium perenne, Festuca arundinaceae and Dactylis glomerata. Among cereals, in Gramineae, the development of a protoplast regeneration system for maize has been especially challenging. Rhodes et al. (1988) were the first to raise protoplast derived maize plantlets from embryogenic cultures which, however, proved sterile. Later, Shillito et al. (1989) initiated embryogenic callus cultures from immature zygotic embryos of an elite inbred maize line B73, which yielded protoplasts that regenerated to form fertile plants. The protoplast-derived plant on crossing with pollen from a seed-derived plant produced viable seeds which germinated normally. Using similar techniques, Prioli and Sondahl (1989) obtained fertile maize plants from embryogenic cell suspension protoplasts of a line (Cat 100-1) adapted to tropical regions.

Embryogenic citrus suspension cultures also provide protoplasts that can be used in the production of interspecific and intergeneric somatic hybrid plants. Equal success can be obtained with protoplasts isolated from nucellus-callus culture of this plant. Ability to isolate protoplasts from embryogenic cultures has had a large impact on their in vitro culture of forest trees, e.g., Pinus taeda, Picea glauca, P. mariana, Pseudotsuga menziesii, Abies alba, Santalum album and Liriodendron tulipfera. Somatic embryos induced on the protoplast-derived calli also germinate to form plantlets which finally establish in the soil.

Genetic Transformation

In seed embryogenesis, zygotic embryos are seated deep inside the nucellar tissue. They live in a protected environment besides being genetically heterogeneous. On the contrary, somatic embryos remain virtually unprotected and more or less give rise to genetically uniform plants. The advent of leaf-disc transformation systems has made it possible to successfully engineer species (Nicotiana tabacum, Medicago sativa) in which, tissues are capable of regeneration via somatic embryogenesis.

In these species, isolated single cells can be transformed in cultures and grown on a selection medium (nutrient medium containing an antibiotic, kanamycin) to callus colonies which eventually form somatic embryos on removal of auxin from this medium (Chabaud et at 1988). Since the callus phase seems essential in this type of indirect somatic embryogenesis, the possibility of chimeric embryos arising from transformed and non-transformed tissues cannot be ruled out. Therefore, the callus phase can be bypassed through a process of repetitive somatic embryogenesis. McGranahan et al. (1990) used repetitive embryogenesis for Agrobacterium-transformed walnut (Juglans regia L.) cells and obtained multiple crops of somatic embryos without employing the callus phase.

There is also evidence to show that repetitive embryos originate from single epidermal or subepidermal cells which can be readily exposed to Agrobacterium. Thus, the transformation technique applied to a primary somatic embryo, instead of a zygotic embryo, should give rise to totally transgenosic somatic embryos. Repetitive embryogenesis is also ideally suited to particle gun-mediated genetic transformation. Instead of relying on Agrobacterium to mediate the transfer of genes into plant cells, the particle gun literally shoots DNA that has been precipitated onto particles of a heavy metal, into the plant cells. Embryogenic suspension cultures of cotton and soybean, initiated from immature embryos, yielded an average of 30 stably transformed cell lines following each firing of the gun. The transformed cell lines can then be induced to form an unlimited number of transformed somatic embryos through repetitive embryogenesis.

Synthesis of Metabolites

The repetitive embryogenesis system is of potential use in the synthesis of metabolites such as pharmaceuticals and oils. Borage (Borage officinalis L.) seeds contain high levels of y-linolenic acid, used as a precursor of postglandins or in the treatment of atopic eczema. Somatic embryos of borage also produce this metabolite but through repetitive somatic embryogenesis a continuous supply of y-linolenic acid is ensured, which otherwise would be limited to the growing season in the zygotic embryo. The same principle can be applied for production in vitro of industrial lubricants from jojoba (Simmondsia chinensis) and leo-palmitostearin (the major ingredient in cocoa butter) from Cacao (Theobroma cacao).

Conclusion

The in vivo clonal propagation of plants is often difficult, expensive and even unsuccessful. Tissue culture methods offer an alternative means of plant vegetative propagation. Clonal propagation through tissue culture (popularly called micropropagation) can be achieved in a short time and space. This is possible through somatic embryogenesis by which rapid formation of embryos takes place leading to multiplication of shoots.

Questions

1. How has somatic embryogenesis helped in clonal propagation of plants?
2. What are Synthetic/ Artificial seeds?
3. Discuss the role of somatic embryogenesis in genetic transformation and protoplast regeneration.

Note